

1st PCR: adding overlaps to dsRed2, c3, c6, c7

	PCR 20 µL reac	Final Concentration		ADN	for primer	rev primer
5X Phusion HF Buffer	4	1X		dsRed2	f_Gbs_dsRed2HH	r_Gbs_HH1
10 mM dNTPs	0,4	200 µM		c3	f_Rmv_c3	r_IDT_dia
10 µM Forward Primer	1	0.5 µM		c6	f_Rmv_c3	r_IDT_cir
10 µM Reverse Primer	1	0.5 µM		c7	f_Gbs_c6c7	r_Gbs_c7ADH1
G-block (0.1ng/µL)	1					
DMSO (optional)	0,6	3 %				
Phusion DNA Polymerase	0,2	1.0 units/50 µl PCR				
Nuclease-free Water	11,8					
Total volume	20					
Number of reactions (including controls):						
WORK ON ICE	DsRed2					
number of reactions:	2				f_primer (1uL of 10 uM)	r_primer (1uL of 10uM)
Mastermix	volume in uL			dsRed2	f_Gbs_dsRed2	r_Gbs_HH1
5X Phusion HF Buffer	8 x			c3_0		
10 mM dNTPs	0,8 x			c3_1		
10 µM Forward primer	2 f_Gbs_DsRed2HH			c3_2		
10 µM Reverse primer	2 r_Gbs_HH1			c3_3		r_IDT_dia
DMSO (optional)	1,2 x			c6_0		
Phusion DNA Polymerase (ADDED LAST)	0,4			c6_1		
Nuclease-free Water	23,6 x			c6_2		
				c6_3	f_Rmv_c3	r_IDT_cir
Volume of master mix in each tube:	19 uL			c7_0		
				c7_1		
				c7_2		
WORK ON ICE	c3			c7_3	f_Gbs_c6c7	r_Gbs_c7ADH1
number of reactions:	5					
Mastermix	volume in uL					
5X Phusion HF Buffer	20					
10 mM dNTPs	2					
10 µM Forward primer	5 f_Rmv_c3					
10 µM Reverse primer	5 r_IDT_dia					
DMSO (optional)	3					
Phusion DNA Polymerase (ADDED LAST)	1					
Nuclease-free Water	59					
Volume of master mix in each tube:	19 uL					
WORK ON ICE	c6					
number of reactions:	5					
Mastermix	volume in uL					
5X Phusion HF Buffer	20					
10 mM dNTPs	2					
10 µM Forward primer	5 f_Rmv_c3					
10 µM Reverse primer	5 r_IDT_cir					
DMSO (optional)	3					
Phusion DNA Polymerase (ADDED LAST)	1					
Nuclease-free Water	59					
Volume of master mix in each tube:	19 uL					
WORK ON ICE	c7					
number of reactions:	5					
Mastermix	volume in uL					
5X Phusion HF Buffer	20					
10 mM dNTPs	2					
10 µM Forward primer	5 f_Gbs_c6c7					
10 µM Reverse primer	5 r_Gbs_c7ADH1					

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DMSO (optional)	3					
Phusion DNA Polymerase (ADDED LAST)	1					
Nuclease-free Water	59					
Volume of master mix in each tube:	19 uL					
PCR programming "YEAST-F":						
Initial Denaturation	98°C	30 seconds				
35 Cycles	98°C	10 seconds				
	58°C	30 seconds				
	72°C	20 seconds				
Final Extension	72°C	10 minutes				
Hold	4°C					